Sera-Mag Select Size Selection and PCR Clean-up Reagent

User Guide

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1 Introduction

About the user guide

- This guide provides information for use of Sera-Mag™ Select reagent for PCR reaction clean-up and DNA size selection. It is valid for the following product codes:
  - 29343045
  - 29343052
  - 29343057
- Tips and FAQs are included to guide the user towards the most satisfactory results.
- A troubleshooting guide is provided to assist in resolving issues that the user may encounter.

Find your local support representative at *cytiva.com/contact*

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

About Sera-Mag Select

Sera-Mag Select reagent for PCR clean-up and size selection is based on the well-known Solid-Phase Reversible Immobilization technology used for selective binding of DNA fragments in applications such as Next Generation Sequencing (NGS) and PCR reaction clean-up.

It combines the ease of magnetic bead technology, using the exceptional binding characteristics of Sera-Mag Select Carboxyl Speedbeads with an optimized binding solution in a ready- to-use formulation. The DNA size range isolated from the process can be tailored to suit user's end requirements by adjusting the amount of reagent that is added to a fixed volume of sample.

As a rule, the smaller the DNA fragment to be bound, the larger the volume of the reagent that should be added (relative to the initial sample volume). DNA of 100 bp size or greater can be reliably recovered using Sera-Mag Select.

2 General tips

- Sera-Mag Select reagent must be stored at 2°C to 8°C.
- Make sure that the product is thoroughly mixed and fully equilibrated to ambient temperature prior to use. We recommend 30-60 minutes (min) continuous mixing on a roller.
- Sera-Mag Select solution exhibits some viscosity. For best reproducibility, we recommend dispensing the reagent by reverse pipetting and removing any excess reagent that might adhere to the outside of the tip (critical in dual side size selection).
- Care should be taken when removing supernatants to prevent accidental aspiration of the beads (addition of 0.1% Tween™-20 should mitigate any potential problems).
- For optimal results a freshly prepared solution of 85% ethanol is recommended for washing steps. Note that the solution must be prepared by mixing appropriate volumes of water and absolute ethanol and not by adding components to the final volume (a mixture of water and ethanol will exhibit a lower total volume than expected).
- For best reproducibility, sample volume should be at least 50 µL.
- Samples must be dissolved in molecular grade water or TE buffer (Tris-EDTA). Note that some of the routinely used laboratory reagents e.g., PEG, glycerol, ethanol etc. will affect size selection.
- For automation, replacing vortexing with mixing by repeated sample pipetting up and down is expected to yield similar results.
- For most applications using solid phase reversible immobilization-based products such as AMPure™ XP and SPRIselect from Beckman Coulter Inc, protocols can be used directly with Sera-Mag Select without need to change. Note that we recommend 85% ethanol for all washing steps.

3 Modes of operation

Sera-Mag Select size selection reagent can be used in the following modes of operation:

- **Recovery mode** which allows the user to bind all DNA in the sample (≥ 100 bp). This mode is used to remove contaminants such as salts, proteins, nucleotides and low molecular weight DNA including adapters or primers. A maximum volume of Sera-Mag Select reagent is applied.
- **Left side size selection** which allows the user to bind DNA fragments above a defined molecular weight using a single adjusted volume of Sera-Mag Select reagent that is less than maximum.
- **Right side size selection** which allows the user to select for fragments below a defined molecular weight. In this mode two sequential volumes of Sera-Mag Select reagent are applied; the first aliquot is used to bind all unwanted DNA which are above a defined molecular weight and the second aliquot which triggers binding of all DNA is the supernatant from step one. (The second volume of the reagent added brings the total reagent volume to maximum).
- **Dual side size selection** which allows the user to select fragments between a defined size range. It combines both right and left side selection to purify DNA fragments centred around a desired size.

3.1 Recovery mode

This mode is typically used for PCR clean-up and DNA concentration, see *Fig. 3.1, on page 7*. The reagent is applied at a single maximum volume to bind all DNA ≥100 bp. For maximum recovery, Sera-Mag Select should be added at 2.5× sample volume. The volume of the reagent can be adjusted according to the amplicon size, e.g., with 500 bp PCR product a ratio of 1x sample volume will allow for maximum recovery.

The following sections describe the recommended protocols for a 50 µL input sample.

Binding/Separation

Step	Action
1	Add Sera-Mag Select reagent at 2.5x sample volume = (2.5 × 50 µL) = 125 µL
2	Vortex the sample for 30-60 seconds (s), briefly spin and incubate at room temperature (RT) for 5-10 min.
3	Place the tube on a magnet rack for 5 min or until the beads have fully settled.
4	Aspirate and discard the supernatant.

Washing beads

Step	Action
1	With the tubes on the magnet, wash twice with 180-200 µL 85% ethanol, do not disturb the bead pellet
2	Take care to aspirate as much wash solution as possible after the second wash.
3	Dry the beads for 5-10 min at RT to remove any residual ethanol.

Elution of bound DNA

Step	Action
1	Add 50 µL of TE buffer, vortex for 30-60 s to resuspend the beads and briefly spin.
2	Incubate for 5-10 min at RT to elute DNA.
3	Return to the magnet for 5 min or until the beads have fully settled.

Step	Action
4	Carefully aspirate the supernatant containing purified DNA and transfer to a fresh tube.

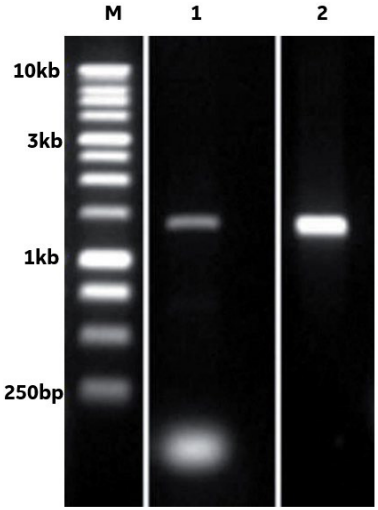

Figure 3.1: ***PCR clean-up****. Agarose gel electrophoresis of PCR sample pre and post clean-up (lane 1 and 2 respectively). The use of Sera-Mag Select reagent successfully removed all primers and allowed for PCR product (~1350 bp) concentration (elution volume < sample input volume)*

3.2 Left side size selection

In this mode, the reagent is applied at a single intermediate level (<2.5x) to bind DNA fragments above a defined threshold. The lower size cut off is defined by the volume of Sera-Mag Select added to the sample. As a rule, the higher the volume of the reagent added, the lower the size cut off. This relationship is illustrated in the Figure below and *Fig. 3.3, on page 9*. Note that the reagent cannot be used at volume ratios below 0.4x. At this level the approximate size cut off is 2.5 kb.

For left side size selection, follow a recovery mode protocol using adjusted volume (V_L) of Sera-Mag Select for binding of DNA above a defined threshold. Use the Figure below and *Fig. 3.3, on page 9* as a guideline for selecting appropriate reagent to sample volume ratios ($0.4x \leq V_L < 2.5x$). For example, if the ratio of 0.8x is chosen for a 50 µL input sample then the volume (V_L) of the reagent to add is: 0.8 × 50 µL = 40 µL.

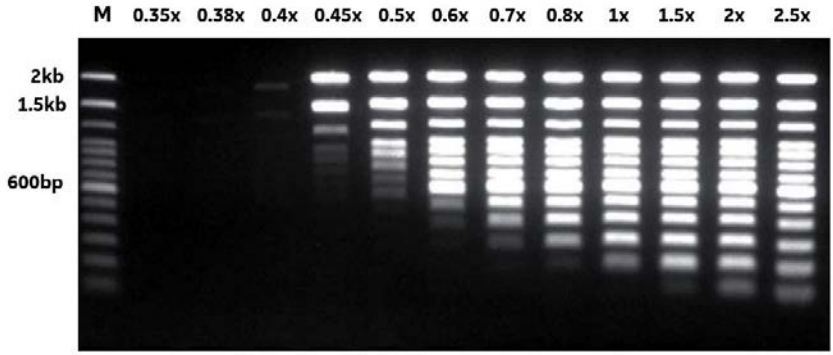

Figure 3.2: ***Left side size selection.*** *DNA ladder was subjected to left side size selection with given ratios of Sera-Mag Select volume to sample volume. Note that isolation of the 2kb fragment is minimal until a ratio of at least 0.45x is used.*

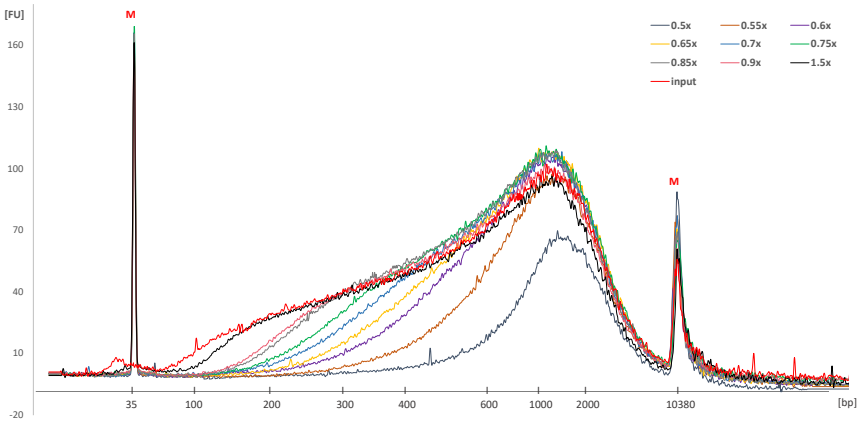

Figure 3.3: ***Left side size selection.*** *Human genomic DNA was fragmented using NEBNext™ dsDNA Fragmentase™ (NEB) and 100 ng of fragmented DNA (50 µL in TE) was subjected to size selection with given ratios of Sera-Mag Select volume to sample volume. DNA was eluted in 50 µL in TE and 1 µl was run on a Bioanalyzer using Agilent™ High Sensitivity DNA Chip.*

3.3 Right side size selection

This mode employs TWO sequential rounds of binding to select fragments below a defined threshold:

- **Round 1:** allows to bind and dispose of unwanted DNA fragments that are above a defined threshold. The desired fraction remains in the supernatant that is retained and processed in round 2. This supernatant contains all the non-binding components from the original sample plus non-bead components of the Sera-Mag Select reagent.
- **Round 2:** equivalent to a single binding round used in the recovery mode at 2.5x sample volume. A fresh aliquot of reagent is added to the maximum volume level to facilitate binding of all DNA in the supernatant from round 1 (≥ 100 bp). The volume needed in round two (V2) can be easily calculated; V2 = 2.5x – V1 (volume added in round 1). After magnetic separation in round 2, the beads contain all desired fragments below a defined threshold.

Following round 2 the beads will contain the inverse content of the left side selection displayed in *Fig. 3.3, on page 9*. Use *Fig. 3.4, on page 12* and *Fig. 3.5, on page 12* as a guideline for selecting appropriate ratios of reagent volume to sample volume for round 1 (0.4x ≤ V1 < 2.5x). For example, for round 1, if the ratio of 0.8x is chosen for 100 µL input sample then the volume of reagent (V1) to add is 0.8 × 100 µL = 80 µL. The volume of the reagent (V2) to add in round 2 is calculated as follows: 2.5x initial sample volume (2.5 x 100 µL = 250 µL) – V1 (80 µL) = 170 µL.

The following sections describe the recommended protocol.

Binding/Separation – Round 1

Step	Action
1	Add Sera-Mag Select reagent at an appropriate sample volume ratio to capture DNA above a defined threshold (0.4x ≤ V1 < 2.5x).
2	Vortex the sample for 30-60 s, briefly spin and incubate at RT for 5-10 min.
3	Place the tube on a magnet rack for 5 min or until the beads have fully settled.
4	Aspirate and RETAIN the supernatant, discard the beads.

Binding/Separation – Round 2

Step	Action
1	Add an additional volume of Sera-Mag Select reagent to trigger maximum DNA binding in the supernatant from round 1 (V2 = 2.5x – V1).

Step	Action
2	Vortex the sample for 30-60 s, briefly spin and incubate at RT for 5-10 min.
3	Place the tube on a magnet rack for 5 min or until the beads have fully settled.
4	Aspirate and DISCARD the supernatant.

Washing beads

Step	Action
1	With the tubes on the magnet, wash twice with 180-200 µL 85% ethanol, do not disturb the bead pellet
2	Take care to aspirate as much wash solution as possible after the second wash.
3	Dry the beads for 5-10 min at RT to remove any residual ethanol.

Elution of bound DNA

Step	Action
1	Add 50 µL of TE buffer, vortex for 30-60 s to resuspend the beads and briefly spin.
2	Incubate for 5-10 min at RT to elute DNA.
3	Return to the magnet for 5 min or until the beads have fully settled.
4	Carefully aspirate the supernatant containing size selected DNA and transfer to a fresh tube.

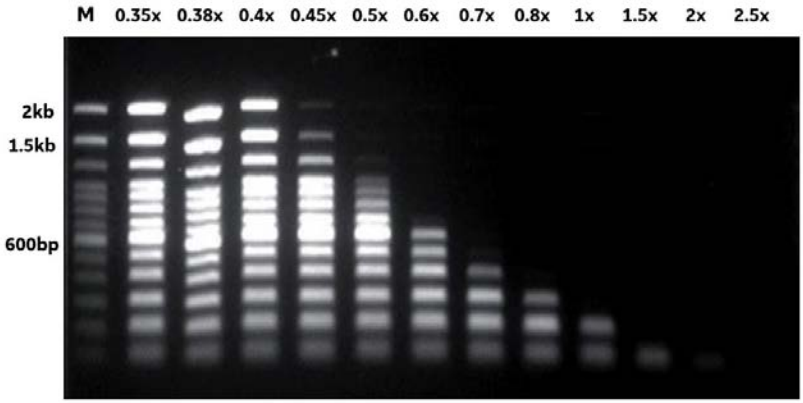

Figure 3.4: ***Right side size selection.*** *DNA ladder was subjected to right side size selection with given ratios of Sera-Mag Select volume to sample volume. Note that right side size selection is essentially the reverse of the left side size selection*

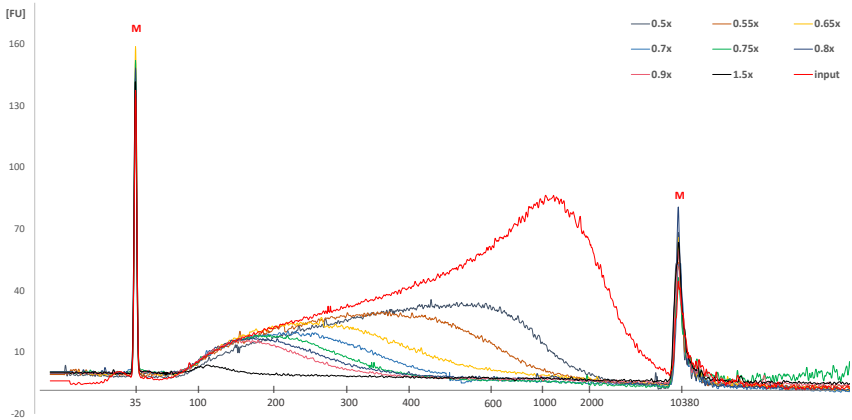

Figure 3.5: ***Right side size selection.*** *Human genomic DNA was fragmented using NEBNext dsDNA Fragmentase (NEB) and 100 ng of fragmented DNA (50 µL in TE) was subjected to size selection with given ratios of Sera-Mag Select volume to sample volume. DNA was eluted in 50 µL in TE and 1 µL was run on a Bioanalyzer using Agilent High Sensitivity DNA Chip.*

3.4 Dual side size selection

This mode uses a sequential combination of both left and right side size selection modes to purify DNA fragments centred around a desired size. This concept is illustrated in *Fig. 3.6, on page 15* and *Table 3.1, on page 15*.

- Round 1: allows to bind and dispose of unwanted DNA fragments that are above a defined threshold. This part is equivalent to the first round of the right size selection and defines the upper size limit.
- Round 2: is performed on the supernatant from round 1 and is equivalent to the left side size selection. A fresh aliquot of reagent is added at the total ratio that is lower than maximum (<2.5x) and set at the level that will define the lower size limit.

After magnetic separation in round 2, the beads contain all desired fragments between a defined size range.

The volume of Sera-Mag Select added in the second binding step can be calculated as follows: $V\underline{2} = V\underline{L} - V\underline{2}$, where $V\underline{L}$ is a total reagent volume needed to obtain left side size selection limit calculated from sample input x desired ratio and $V\underline{1}$ is a volume added in round 1 calculated from sample input x desired right side selection ratio. For example, if the ratio of 0.9x has been chosen for a left side size limit and a ratio of 0.6x has been chosen for a right side selection limit for an input sample of 100 µL, then the volumes used are as follows: $V\underline{1}$ (0.6 x 100 µL) = 60 µL; $V\underline{L}$ (0.9 x 100 µL) = 90 µL, $V\underline{2} = (V\underline{L})$ 90 µL – $(V\underline{1})$ 60 µL = 30 µL

The accuracy of the first-round size selection is critical for later steps; any aspiration of the beads will result in larger fragments being carried over into final fragment pool. Any loss (suboptimal aspiration) of supernatant will affect the size selection in round 2 due to the altered total ratio of reagent volume to sample volume.

As a rule, the left side size selection ratio (round 2) is always higher that the right side size selection ratio (round 1). Note that a very narrow size selection comes at a cost of reduced yield.

The following sections describes the recommended protocol.

Binding/Separation – Round 1

Step	Action
1	Add Sera-Mag Select reagent at an appropriate sample volume ratio to capture DNA above a defined threshold ($0.4x \leq V\underline{1} < 2.5x$).
2	Vortex the sample for 30-60 s, briefly spin and incubate at RT for 5-10 min.
3	Place the tube on a magnet rack for 5 min or until the beads have fully settled.
4	Aspirate and RETAIN the supernatant, discard the beads.

Binding/Separation – Round 2

Step	Action
1	Add an additional volume of Sera-Mag Select reagent to the supernatant from round 1 to allow for binding of DNA fragments above a defined threshold (V2 = VL – V1).
2	Vortex the sample for 30-60 s, briefly spin and incubate at RT for 5-10 min.
3	Place the tube on a magnet rack for 5 min or until the beads have fully settled to the magnet
4	Aspirate and DISCARD the supernatant.

Washing beads

Step	Action
1	With the tubes on the magnet, wash twice with 180-200 µL 85% ethanol, do not disturb the bead pellet
2	Take care to aspirate as much wash solution as possible after the second wash.
3	Dry the beads for 5-10 min at RT to remove any residual ethanol.

Elution of bound DNA

Step	Action
1	Add 50 µL of TE buffer, vortex for 30-60 s to resuspend the beads and briefly spin.
2	Incubate for 5-10 min at RT to elute DNA.
3	Return to the magnet for 5 min or until the beads have fully settled.
4	Carefully aspirate the supernatant and transfer to a fresh tube.

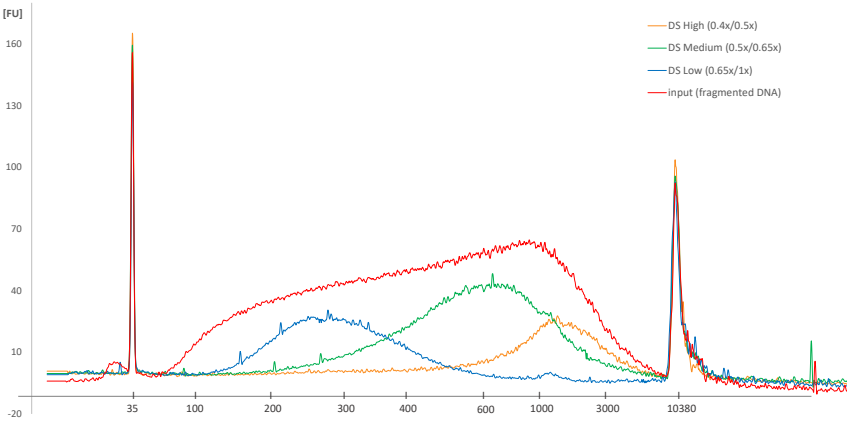

Figure 3.6: ***Dual side size selection.*** *Human genomic DNA was fragmented using NEBNext dsDNA Fragmentase (NEB) and 100 ng of fragmented DNA (50 µL in TE) was subjected to size selection with given ratios of Sera-Mag Select volume to sample volume. DNA was eluted in 50 µL in TE and 1 µL was run on a Bioanalyzer using Agilent High Sensitivity DNA Chip*

Table 3.1: Dual side size selection.

Average fragment size	Approximate size distribution	Ratio (right-left)	Round 1 ratio (right side size limit)	Sera-Mag Select round 1 volume (V1)	Round 2 ratio (left side size limit)	Sera-Mag Select round 2 volume (V2)
250 bp	150 – 500 bp	0.65x/1x	0.65x	65 µL	0.35x (1x total)	35 µL
380 bp	200 – 600 bp	0.6x/ 0.825x	0.6x	60 µL	0.225x (0.825x total)	22.5 µL
390 bp	200 – 700 bp	0.585x/ 0.85x	0.585x	58.5 µL	0.265x (0.85x total)	26.5 µL
400 bp	180 – 800 bp	0.575x/ 0.9x	0.575x	57.5 µL	0.325x (0.9x total)	32.5 µL

Average fragment size	Approximate size distribution	Ratio (right-left)	Round 1 ratio (right side size limit)	Sera-Mag Select round 1 volume (V1)	Round 2 ratio (left side size limit)	Sera-Mag Select round 2 volume (V2)
400 bp	220 – 900 bp	0.57x/ 0.78x	0.57x	57 µL	0.21x (0.78x total)	21 µL

Exemplary ratios and volumes used for dual size selection of fragmented DNA for recovery of specific fragments based on 100 µl input sample . Please note that the ratios are a guideline only and size selection might be affected by the composition of the input sample and may require optimisation.

4 FAQS and tips

1. **Can Sera-Mag Select magnetic particles be left in PCR reactions?**

   No, ensure that no magnetic particles are carried over into the PCR reaction as this may impact downstream performance.

2. **Can I use Sera-Mag Select for both clean up and size selection?**

   Yes, Sera-Mag Select is manufactured to standards suitable for both PCR Clean-up and size selection for which, protocols are provided.

3. **Will the size selection remove all primers and adapters from the PCR reaction?**

   The standard recovery mode will effectively eliminate all primers and adapters from the PCR reaction provided they are below 100 bp. In case of adapters having a higher molecular range, complete removal can be accomplished using adjusted (<2.5x) volume of the reagent (left side size selection protocol).

4. **What is the smallest fragment I can select for in my reaction?**

   The smallest fragment size is 100 bp, please refer to the size selection protocol.

5. **Can I use Sera-Mag Select to purify genomic DNA?**

   Sera-Mag Select will bind genomic DNA but is not designed for this purpose. Extremely large DNA strands can prove difficult to elute and may bind across magnetic particles causing them to aggregate.

6. **Do I need to change the program on my liquid handler if I am currently using AMPure XP beads?**

   For most applications, Sera-Mag Select can normally be substituted directly for AMPure XP without any change to the existing script(s), however for optimal yield we recommend using 85% ethanol in place of 70% ethanol. In some applications where a very tight size selection is desired, additional optimization might be needed.

7. **Is tighter size selection important?**

   Selecting fragments in a narrow range will negatively affect total recovery. However, in some applications, a very narrow fragment range is required. Follow advice specific for downstream application in which the size selected DNA is to be used.

8. **What is the upper size limit for dual side size selection?**

   The upper size limit for dual size selection is around 2 kb meaning that it is impossible to perform dual size selection on DNA fragments above 2 kb.

9. **What are the proper storage conditions for the Sera-Mag Select?**

   We recommend refrigeration between 2°C to 8°C when not in use, do not freeze. Store upright and kept the bottle tightly sealed. Avoid prolonged exposure to RT.

10. **Can I leave Sera-Mag Select at room temperature and for how long?**

We only recommend 2°C to 8°C storage. Avoid prolong exposure at RT.

11. **How long must I equilibrate at room temperature before using the Sera-Mag Select beads?**

    Depending on the bottle size, this will be a minimum of 30 minutes at room temperature, for best performance ensure that the beads are fully equilibrated before use.

12. **How long should I mix the Sera-Mag Select beads before adding them to my sample?**

    We recommend mixing until the solution is visibly homogeneous.

13. **What is the best way to mix the Sera-Mag Select beads?**

    We recommend mixing the beads on a roller mixer as this will help the product to equilibrate to RT faster. Other mixing methods such as gentle repeated inversion or gentle vortexing are also acceptable.

14. **Can the Sera-Mag Select be frozen?**

    Do not freeze as it may impact the performance of the product.

5 Troubleshooting

Issue	Cause	Solution
No or very low DNA recovery	Reagent is non-homogeneous: magnetic beads have settled out of suspension during shipment or storage. Reagent has not been equilibrated to room temperature before use.	Make sure the reagent is fully homogenized and equilibrated before use. Make sure that sample does not contain components affecting DNA recovery
Inconsistent DNA recovery	Loss of magnetic beads during workflow: incomplete settling to magnets or/and accidental aspiration of the beads	Make sure the beads are fully settled to the magnet before aspirating the supernatant. Avoid disturbing bead pellets during washing steps (should this happen make sure the beads have resettled before continuing). Inadequate magnetic separation; use an alternative device. Include a surfactant such as Tween-20 at 0.1% with input sample to reduce surface tension.

Issue	Cause	Solution
Inconsistent size selection profile	Poor precision of sample and/or reagent volumes. Size selection profile is very sensitive to relative volumes at higher size cut offs.	Make sure liquid dispenser (e.g., pipette) is properly calibrated. Use reverse pipetting action to counter viscosity of the reagent. For manual operations, make sure that excess reagent that may adhere to the outside of the pipette tip is removed before dispensing. Make sure that sample components are not affecting size selection profile (re-adjust the ratio used).
DNA recoveries decreasing over time	Reagent degradation (microbial contamination or/and inappropriate storage conditions).	Avoid prolong exposure to RT. Consider using a smaller unit size or aliquoting the reagent.
Low purity of recovered DNA	Recovered DNA is contaminated with non-bead components or ethanol.	Make sure there is no residual ethanol associated with the beads before proceeding with elution (aspirate all ethanol and make sure the beads are dried for 5-10 min). Make sure that the binding mix suspension is settled to a single volume after vortexing by a brief centrifugation (this will prevent any carry-over of sample components). It can be necessary to increase the volume of the wash solution.

6 Related products

Item	Product code	Image
MagRack 6	28948964	
MagRack Maxi	28986441	

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